Electronic Version
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Claims

[c1] 1)A method of concentrating and extracting particles from a blood sample, the method comprising:
exposing the blood sample to an enzyme-detergent combination; and
analyzing the exposed blood sample for the presence of particles.

[c2] 2)The method of claim 1 wherein the enzyme-detergent combination comprises plasminogen and streptokinase.

[c3] 3)The method of claim 2 further comprising the steps of:
freezing the plasminogen and streptokinase in coincident relation until a fibrin lysis reagent is needed; and
reacting streptokinase with plasminogen upon thawing whereby plasmin is formed.

[c4] 4)The method of claim 2 further comprising the step of suspending the plasminogen in an aqueous salt solution prior to freezing.

[c5] 5)The method of claim 4 wherein the aqueous salt solution comprises NaCl.

[c6] 6)The method of claim 4 wherein the aqueous salt solu-

tion comprises $NaPO_4$.

[c7] 7)The method of claim 1 wherein the particles are selected from a group consisting of prions, toxins, metabolic markers, cancerous matter, disease state markers, bacteria, virus, and fungi.

[c8] 8)The method of claim 1 further comprising the step of replicating the particles through PCR.

[c9] 9)The method of claim 1 further comprising the step of introducing DNase to the blood sample.

[c10] 10)The method of claim 1 further comprising the step of introducing Endonuclease to the blood sample.

[c11] 11)The method of claim 2 wherein the plasminogen and streptokinase are in a dried state.

[c12] 12)The method of claim 11 wherein the plasminogen and streptokinase are mixed and distributed in disposable test containers.

[c13] 13)The method of claim 11 wherein the plasminogen is combined with Phospholipase A_2,DNase, Endonuclease, and Lipase.

[c14] 14)The method of claim 13 wherein the enzyme-detergent combination is suspended then dried in pellets

of trehalose buffer and packaged as a dry reagent.

[c15] 15)The method of claim 11 wherein the streptokinase is suspended then dried in pellets of trehalose buffer and packaged into tubes as a dry reagent.

[c16] 16)The method of claim 11 further comprising:
resuspending the dried reagents in a buffer;
adding the solution to the volume of blood; and
incubating the sample for at room temperature.

[c17] 17)The method of claim 16, wherein the dried reagent is comprised of 1,500-4,500 KU Phospholipase A_2, 5,000-10,000 U Streptokinase, 2-10 U Plasminogen, 200-3,650 U DNase, 200-4,000 U Endonuclease, and 10,000-100,000 Lipase.

[c18] 18)The method of claim 16 further comprising:
centrifuging the solution;
decanting the supernatant; and
washing the pellet.

[c19] 19)The method of claim 18 wherein the solution is centrifuged for approximately 20 minutes at 5,000-5,500 x g at a temperature of 10-20°C.

[c20] 20)The method of claim 18 wherein the pellet is washed with an Ecotine-HEPES solution.

[c21] 21)The method of claim 18 wherein the pellet is washed with a Sucrose-HEPES solution.

[c22] 22)The method of claim 18 wherein the pellet is washed with an Ecotine-HEPES solution and a Sucrose-HEPES solution.

[c23] 23)The method of claim 16 further comprising:
centrifuging the solution;
decanting the supernatant;
digesting the sample; and
applying the sample to a commercially available nucleic acid extraction method.

[c24] 24)The method of claim 23 wherein digesting the sample further comprises lysis and DNase inactivation.

[c25] 25)The method of claim 23 wherein digesting the sample further comprises lysis and Endonuclease inactivation.

[c26] 26)The method of claim 23 wherein digesting the sample further comprises utilizing proteinase K, sodium dodecyl sulfate, aurintricarboxylic acid, and sodium citrate buffer, incubated at room temperature.

[c27] 27)The method of claim 16 further comprising:
filtering the solution;
washing the solution;

digesting the sample; and
purifying the extract through commercially available methods.

[c28] 28)The method of claim 27 wherein digesting the sample further comprises lysis and DNase inactivation.

[c29] 29)The method of claim 27 wherein digesting the sample further comprises lysis and Endonuclease inactivation.

[c30] 30)The method of claim 27 wherein digesting the sample further comprises the steps of:
combining proteinease K, aurintricarboxylic acid, and sodium citrate buffer;
incubating at room temperature; and
eluting the lysate from the filter surface.

[c31] 31) The method of claim 16 further comprises applying the solution directly to a biosensor device whereby responsive to the presence of the pathogens in the blood sample, the patient develops pathogenic or native disease state markers which allow for the capture and detection of these markers by the biosensor device.

[c32] 32)The method of claim 16 further comprises applying the solution directly to a liquid chromatography mass spectrometry device whereby, responsive to the presence of the pathogens in the blood sample, the patient devel-

ops pathogenic or native disease state markers that allow for the detection of mass signatures associated with the structural components of the pathogens using the mass spectrometry device.

[c33] 33)The method of claim 16 wherein the buffer comprises Potassium Phosphate, Magnesium Chloride, Sodium Chloride, and Aurintricarboxylic Acid.

[c34] 34) The method of claim 33 wherein the buffer further comprises Triton X-100.

[c35] 35) The method of claim 33 further comprising the step of storing the enzymes with a trehalose buffer.

[c36] 36) The method of claim 35 further comprising the step of combining methyl 6-O-(N-heptylcarbamoyl)-α-D-glucopyranoside and Saponin in the trehalose buffer.

[c37] 37) The method of claim 36 wherein a concentration of 20-35 mM of methyl 6-O-(N-heptylcarbamoyl)-α-D-glucopyranoside is used.

[c38] 38)The method of claim 36 wherein a concentration of 0.05-0.1% Saponin is used.

[c39] 39) The method of claim 35 wherein the trehalose storage buffer comprises Potassium Phosphate, Triton X-

100, Dithiothreitol, and Trehalose.

[c40] 40) The method of claim 39 wherein the trehalose storage buffer comprises 10 mM Potassium Phosphate.

[c41] 41) The method of claim 39 wherein the trehalose storage buffer comprises 0.01-0.04% Triton X-100.

[c42] 42) The method of claim 39 wherein the trehalose storage buffer comprises 1-5 mM Dithiothreitol.

[c43] 43) The method of claim 39 wherein the trehalose storage buffer comprises 0.3-0.5 M Trehalose.